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Differential Nuclear and Cytoplasmic Expression of PTEN in Normal Thyroid Tissue, and Benign and Malignant Epithelial Thyroid Tumors

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Germline mutations in *PTEN* (*MMAC1/TEP1*) are found in patients with Cowden syndrome, a familial cancer syndrome which is characterized by a high risk of breast and thyroid neoplasia. Although somatic intragenic *PTEN* mutations have rarely been found in benign and malignant sporadic thyroid tumors, loss of heterozygosity (LOH) has been reported in up to one fourth of follicular thyroid adenomas (FAs) and carcinomas. In this study, we examined PTEN expression in 139 sporadic nonmedullary thyroid tumors (55 FA, 27 follicular thyroid carcinomas, 35 papillary thyroid carcinomas, and 22 undifferentiated thyroid carcinomas) using immunohistochemistry and correlated this to the results of LOH studies. Normal follicular thyroid cells showed a strong to moderate nuclear or nuclear membrane signal although the cytoplasmic staining was less strong. In FAs the neoplastic nuclei had less intense PTEN staining, although the cytoplasmic PTEN-staining intensity did not differ significantly from that observed in normal follicular cells. In thyroid carcinomas as a group, nuclear PTEN immunostaining was mostly weak in comparison with normal thyroid follicular cells and FAs. The cytoplasmic staining was more intense than the nuclear staining in 35 to 49% of carcinomas, depending on the histological type. Among 81 informative tumors assessed for LOH, there seemed to be an associative trend between decreased nuclear and cytoplasmic staining and 10q23 LOH ($P = 0.003$, $P = 0.008$, respectively). These data support a role for PTEN in the pathogenesis of follicular thyroid tumors. *(Am J Pathol 2000, 156:1693–1700)*

The tumor suppressor PTEN, also known as MMAC1 and TEP1,[1–3] has recently been shown to play an important role in the pathogenesis of a variety of human cancers.[1,2,4] *PTEN* is located on chromosome subband 10q23.3[2,5] and encodes a dual-specificity phosphatase with lipid and protein phosphatase activity. The major substrate for PTEN is phosphatidylinositol-3,4,5-trisphosphate, a direct product of phosphoinositol-3-kinase activity.[6–8] Phosphatidylinositol-3,4,5-trisphosphate mediates growth factor-induced activation of intracellular signaling, in particular through the serine-threonine kinase Akt (also referred to as Akt1, RAC1, or PKB), which is known to promote cell survival and cell proliferation. High levels of PTEN are associated with low levels of phosphorylated Akt which leads to the induction of apoptosis; hence, loss of PTEN function leads to increased activity of Akt and subsequently cell survival.[7–10] PTEN may also affect other pathways, such as the focal adhesion kinase and mitogen activated protein kinase pathways.[11,12] In other words, PTEN has been shown to mediate G1 cell-cycle arrest and/or apoptosis via the phosphoinositol-3-kinase-Akt pathway in several cell lines such as glioma, breast, and prostate cell lines.[13–15] PTEN therefore seems to play an important role in cell cycle growth, migration, and death. Germline *PTEN* mutations have been identified in the autosomal dominant

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hamartoma Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome.[16,17] Benign thyroid disease is characteristic of both CS and Bannayan-Riley-Ruvalcaba syndrome.[18] The risk of nonmedullary thyroid carcinomas is increased in CS.[19–21] In humans, these thyroid cancers are most often follicular and very rarely papillary thyroid carcinoma (PTC). In contrast, histopathological analyses of *Pten* ± chimeric and heterozygous mice showed lesions consistent with well-differentiated PTC[22] as well as follicular or papillary thyroid neoplasia.[23] Although LOH (loss of heterozygosity) on chromosome band 10q23 has been identified in ~26% of follicular thyroid adenomas (FAs)[24–26] and up to 27% of FTCs,[27] somatic intragenic mutations of *PTEN* are rare.[25,27] The human syndromic and murine data for PTEN involvement in thyroid neoplasia is strong. In accordance with the Knudson "two hit" hypothesis of carcinogenesis, if a somatic mutation is found on one allele of *PTEN* and LOH or a deletion is found on the opposite allele, then biallelic inactivation of *PTEN* at the structural level is said to occur. However, to date, all genetic studies of *PTEN* in human primary thyroid tumors have only demonstrated monoallelic structural mutation (either a heterozygous deletion or a single-hit somatic intragenic mutation). Whether PTEN inactivation at the protein level or via other mechanisms apart from structural alteration applies to thyroid tumorigenesis is unknown. Thus, we sought to determine whether functional biallelic inactivation of PTEN occurs in sporadic nonmedullary thyroid adenomas and carcinomas by examining them for PTEN expression using immunohistochemistry in conjunction with LOH analysis.

Materials and Methods

Thyroid Samples

Paraffin blocks from 139 unselected benign and malignant nonmedullary thyroid tumors were ascertained from Germany, Australia, and Switzerland. Histological classification of the thyroid tumors was in accordance with the World Health Organization.[28] Of note, five papillary tumors were classified as follicular type (Lindsay tumor), and 13 tumors (seven FAs, five FTCs, one PTC) had a prominent granular eosinophilic-appearing cytoplasm (also known as oxyphilic or Hürthle cell).

Anti-PTEN Antibody Specificity

The monoclonal antibody 6H2.1 raised against the last 100 C-terminal amino acids of PTEN[29] was used in all immunohistochemical analyses. As biochemical proof of antibody specificity for PTEN, total protein lysates were obtained[10,15] from a series of thyroid cell lines for which PTEN status is known: NPA-87, K-1, FTC-133, and WRO-82–1 (gifts from D. V. Canlapan and D. Wynford-Thomas). Further, as an additional positive control, the wild-type full-length human *PTEN* cDNA sequence was cloned into the expression vector pcDNA3 and transfected into the PTEN null line FTC-133. Western blot analysis was performed as previously described[10] except that 6H2.1 was used at a 1:250 dilution. Thyroid lines with endogenously expressing or exogenously introduced PTEN all demonstrated a single band at 55 kd, the molecular weight predicted for PTEN, although the PTEN null lines did not cross-react with 6H2.1. No other nonspecific bands were noted, thus proving antibody-specificity (Figure 1). Control antibody against α-tubulin (Sigma, St. Louis, MO), used at 1:10,000 dilution, immunoreacted evenly across protein lysates from all cell lines (Figure 1). The specificity of the antibody 6H2.1 and its suitability for immunohistochemistry in paraffin-embedded tissue has been demonstrated previously.[29] In brief, we used the antibody against embedded *PTEN*-transfected U2OS cells, BALBc/3T3, Nalm6, and DU145 as positive controls; MDA-MB-468 with hemizygous deletion of *PTEN* and a truncation of the remaining allele; A172 which has loss of one *PTEN* allele, and a truncating mutation in exon 2 of the remaining allele; and PC3, which is null for PTEN.[29] Further, commercially available peptide corresponding to PTEN has been used to successfully compete away 6H2.1 immunostaining in paraffin-embedded tissue (GL M, unpublished data).

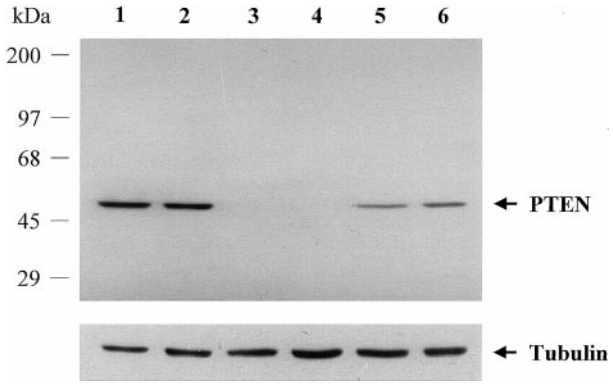


Figure 1. Western analysis of whole-cell protein lysates from thyroid cancer cell lines using the anti-PTEN monoclonal antibody 6H2.1 (**top panel**) and using the anti-α-tubulin antibody as a control (**bottom panel**). NPA-87 and K-1, which are two PTC lines, and WRO-82–1, a FTC line, have endogenous PTEN. FTC-133 is a FTC line that is PTEN null. **Lane 1,** NPA-87; **lane 2,** K-1; **lane 3,** FTC-133; **lane 4,** FTC-133 transfected with empty vector; **lane 5,** FTC-133 transfected with vector containing PTEN; **lane 6,** WRO-82–1. The same membrane was used for Western blot with 6H2.1 as well as anti-tubulin antibody.

Recently, it has been shown that a processed *PTEN* pseudogene (psi*PTEN*) can be transcribed in a number of cell lines and tissue types.[30] For this reason, RNA *in situ* hybridization is not reliable. Psi*PTEN*, however, does not seem to be translated, at least in thyroid tumors, and therefore it would not be expected to complicate the analysis in this study.

Immunohistochemistry

The tissue samples were fixed by immersion in 10% buffered formalin and subsequently embedded in paraffin according to standard protocols. Four-mm sections were cut, mounted on Superfrost plus slides, and baked for 2 hours at 60°C. Subsequently, the sections were deparaffinized and rehydrated by passing through xylene and a graded series of ethanol solutions. Antigen

retrieval was performed by boiling at 98°C in 0.01 mol/L sodium citrate buffer, pH 6.4, in a microwave oven for 20 minutes. To block endogenous peroxidase activity, the sections were incubated with 0.3% hydrogen peroxide in methanol for 30 minutes after cooling to room temperature. After blocking for 30 minutes in 0.75% horse serum, the sections were incubated with a PTEN monoclonal antibody 6H2.1 (dilution 1:100) for 1 hour at room temperature. Primary antibody binding was localized by using an avidin-biotin-peroxidase kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's instruction. The chromogenic reaction was carried out with 0.05% 3,3′diaminobenzidine (Sigma, St. Louis, MO) using nickel cobalt amplification which gives a black product.[31] After counterstaining with Nuclear Fast Red (Rowley Biochemical Institute, Danvers, MA) and mounting, the slides were independently evaluated under a light microscope by two investigators (OG and AP) and randomly spot evaluated by a third investigator (CE). Intensity of staining was classified separately for the nucleus/nuclear membrane and the cytoplasm and graded strong (+++), moderate (++), weak (+), or absent (−). These independent assessments did not differ by more than one grading level.

LOH Analysis

In 95 samples, tumor tissue and blood or corresponding normal tissue (either normal thyroid tissue or adjacent muscle tissue) were available for extraction of paired somatic and germline DNA to study LOH. DNA extraction after microdissection was performed using standard protocols.[32] All subsequent polymerase chain reactions were carried out using 0.6 μM each of forward and reverse primer in 1× polymerase chain reaction buffer (Qiagen, Valencia, CA), 4.5 mmol/L $MgCl_2$ (Qiagen), 1× Q-buffer (Qiagen), 2.5 U HotStarTaq polymerase (Qiagen), and 200 μmol/L dNTP (Gibco, Gaithersburg, MD) in a final volume of 50 μl. Reactions were subjected to 35 cycles of 94°C for 1 minute, 55°C to 60°C for 1 minute, and 72°C for 1 minute followed by 10 minutes at 72°C. Potential hemizygosity at the *PTEN* locus was assessed by screening for a T/G polymorphism within *PTEN* intron 8 (IVS8 + 32G/T) detected by differential digestion with the restriction endonuclease *Hinc*II as previously described[25] except for using the primers PTEN-E8-F (5′-GCGTGCAGATAATGACAAGG-3′) and PTEN-I8-R (5′-TGTCAAGCAAGTTCTTCATCG-3′). If the result of the digestion was not informative, LOH analysis was performed using markers flanking *PTEN*, D10S541 (telomeric) and D10S579 (centromeric)[1,16] as well as the marker D10S2491 that lies within *PTEN*.[33] All forward primers were 5′-labeled with either HEX or 6-FAM fluorescent dye (Research Genetics, Huntsville, AL). Polymerase chain reactions were carried out as described above and separated by electrophoresis through 6% denaturing polyacrylamide gels using an Applied Biosystems model 377 automated DNA sequencer (Applied Biosystems, Perkin-Elmer Corp., Norwalk, CT). The results were analyzed by automated fluorescence detection using the GeneScan collection and analysis software (GeneScan, Applied Biosystems). Scoring of LOH was performed by inspection of the GeneScan analysis output. A double peak, observed in the microsatellite marker that was amplified from DNA extracted from the germline sample, indicated heterozygosity. A single peak in DNA extracted and amplified from the corresponding tumor sample indicated a loss of one allele. If normal cells were admixed with tumor cells, a ratio of 1.5:1 or greater of germline DNA peak to tumor DNA peak was also considered LOH.[24]

To examine the correlative trend between PTEN staining intensity and LOH, we performed a Mantel-Haenszel test[34] for trend in the association between the row and column variables. A $P < 0.05$ was considered statistically significant.

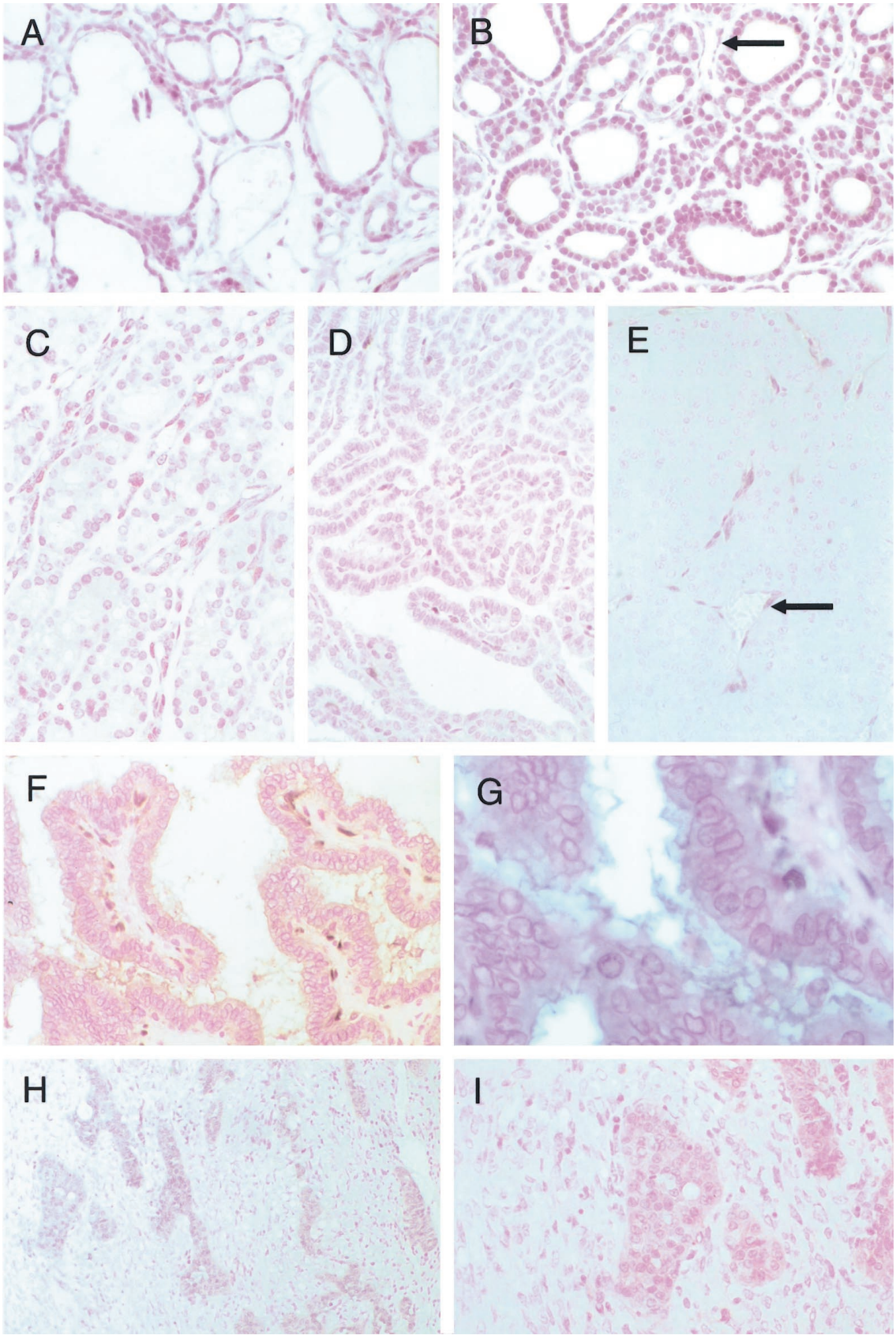
Results

PTEN Immunohistochemistry in Normal Thyroid and Primary Thyroid Tumors

Of the total 139 thyroid tumor samples examined for PTEN expression using the monoclonal antibody 6H2.1, 50 had accompanying normal thyroid tissue. Normal follicular thyroid cells showed a uniform strong (+++) to moderate (++) nuclear or nuclear membrane (hereafter referred to as nuclear) signal whereas the cytoplasmic staining was less strong, + to ++ (Figure 2A). Endothelial cells showed strong (+++) to moderate (++) PTEN expression with a nuclear predominance and were useful as internal positive controls (Figure 2, B and E). In contrast, nuclear and cytoplasmic staining intensity of fibrocytes was very heterogeneous and varied from weak (Figure 2A) to strong (Figure 2F).

The quality and intensity of PTEN immunostaining in the nucleus and cytoplasm in 132 of 139 thyroid tumors was relatively uniform throughout each specimen. However, in seven carcinomas, PTEN expression differed significantly within different regions of each tumor (see below). Because PTEN expression in each of these seven tumors could not be classified into a single category, these different regions were classified separately as if they were two separate tumors, ie, PTEN expression was classified in 139 + 7 = 146 thyroid tumors. Hence, the intensity of PTEN staining in the nucleus and cytoplasm were assessed for 146 thyroid tumors (Figure 3) for purposes of this study.

In FA, the neoplastic nuclei had less intense PTEN immunostaining (+ to ++) compared to normal follicular epithelium whereas the cytoplasmic PTEN staining intensity did not differ significantly from that observed in normal follicular cells (Figures 2B and 3). In thyroid carcinomas (FTC, PTC, and undifferentiated thyroid carcinoma [UTC]) as a group, nuclear PTEN immunostaining was mostly weak in comparison with normal thyroid follicular cells and FAs. Among the three classes of carcinomas, FTCs had the strongest immunostaining in both the nucleus and cytoplasm and UTCs the weakest (Figures 2, C–E, and 3). A few carcinomas, in particular UTCs,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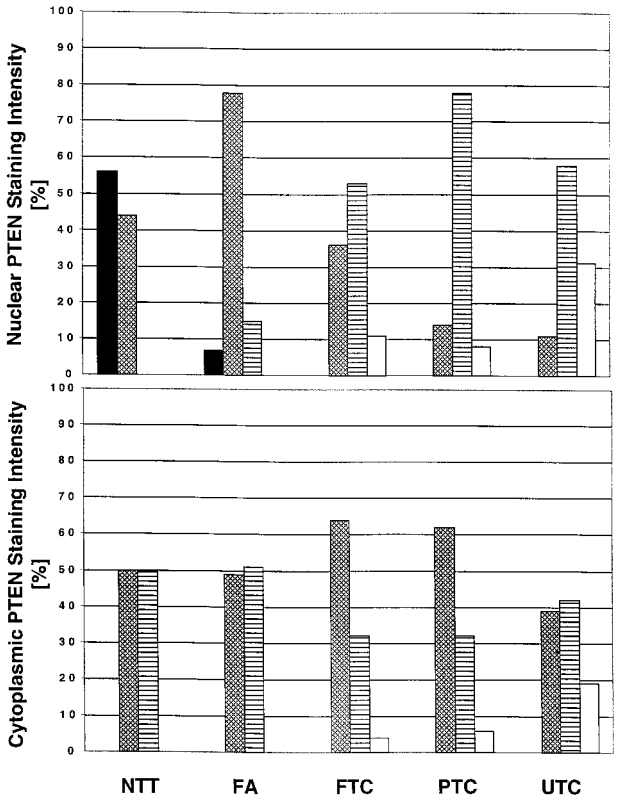

Figure 3. Distribution of PTEN immunostaining intensity in and cytoplasm in thyroid samples (50 normal thyroid tissues, 55 thyroid adenomas, 28 FTC, 37 PTC, and 26 UTC). Strong ■, moderate ▨, weak ▤, and absent staining □. NTT, normal thyroid tissue. The *y*-axis represents percentage of samples with various intensities of nuclear (**top**) or cytoplasmic (**bottom**) staining.

showed no PTEN staining in the nucleus (FTC, 3 of 28; PTC, 3 of 37; UTC, 8 of 26) or in the cytoplasm (one FTC, two PTCs, five UTCs; Figure 2E); eight carcinomas (one FTC, two PTCs, five UTCs) had no immunoreactivity in the nucleus or in the cytoplasm. In almost half of all thyroid carcinomas (FTC, 46%; PTC, 49%; UTC, 35%), the cytoplasmic staining was more intense than the nuclear staining (Figures 2, F and G, and 3). In contrast, the more intense cytoplasmic over nuclear staining was observed in only 7% of FAs. In other words, the stepwise decrease in PTEN immunoreactivity in the nucleus seemed to precede that in the cytoplasm from normal thyroid tissue to FA to carcinomas and finally to UTC (Figure 3). There was no obvious difference in PTEN staining pattern and intensity in Hürthle cell tumors compared to non-Hürthle cell tumors. Fibrocytes seemed to stain a little more intensely in tumor stroma (Figure 2F) compared to normal thyroid stroma (Figure 2A).

Table 1. LOH Analysis of Markers on Chromosome Band 10q23 in 92 Informative Thyroid Tumors

Tumor type	LOH 10q23	%	% of LOH literature*
Follicular thyroid adenoma	8/41	20	6–26 (10q23)
Follicular thyroid carcinoma	6/20	30	7–27 (10q23)
Papillary thyroid carcinoma	3/14	21	5 (10q)
Undifferentiated thyroid carcinoma	10/17	59	35 (10q)

*Data based on References 24, 25, 27, 44, and 45.

Seven carcinomas (four UTCs, two PTCs, one FTC), but no adenomas, showed dichotomous regional PTEN staining within each sample. These were characterized either by islands of strongly immunopositive cells among sheets of cells staining more weakly (Figure 2, H and I) or by single cells staining weakly randomly distributed among cells staining strongly. In one UTC, the positive cells (graded +) were small and more regular whereas the PTEN-negative cells were larger, pleiomorphic, and had a more undifferentiated appearance. This correlation, however, was not seen in the other three UTCs. This pattern of immunostaining in the former UTC was replicated several times, thus indicating that this was not an artifact.

Comparison of Immunohistochemical and LOH Data

In 95 tumors, normal and tumor tissue samples were available for LOH analysis. Similar to the immunohistochemical analysis, the four available carcinomas with dichotomous staining intensity within each sample, which had paired normal and tumor tissue, were considered as eight samples. Therefore, 99 total samples were considered. For purposes of this study, which was to compare the immunohistochemical data to the LOH data, one copy of *PTEN* was considered to be physically deleted only when one or more of the markers, which lie within or closely flanking the gene, showed LOH. Using this definition for monoallelic *PTEN* deletion, LOH data from 92 tumors were informative. Of the 92 informative tumors, 27 tumors (29%) were shown to have loss of one allele of *PTEN* and 65 tumors were classified as having no LOH (Table 1).

LOH analysis was performed separately for regions with different staining intensity in those four carcinomas

Figure 2. Immunohistochemical analysis of normal thyroid, FA, FTC, PTC, and UTCs using the anti-PTEN monoclonal antibody 6H2.1. **A:** Normal thyroid tissue (magnification, ×20). Note strong (+++) to moderate (++) nuclear and weak (+) cytoplasmic staining in the follicular epithelial cells. **B:** Follicular thyroid adenoma (magnification, ×20). In this particular sample, the intensity of nuclear PTEN staining in the majority of follicular cells is strong (graded +++ to ++); the intensity of cytoplasmic staining is weak (+), not greatly different from that of normal epithelial cells. Note the strong staining intensity of the endothelial cells that serve as positive controls (←). **C:** FTC (magnification, ×20) with mainly weak (+) nuclear staining. **D:** PTC (magnification, ×20) with weak (+) nuclear staining. **E:** UTC (magnification, ×20) with absent (−) nuclear and cytoplasmic staining. Note the intense immunostaining in the endothelial cells, which serve as an internal positive control (←). **F:** PTC (magnification, ×20) with absent (−) nuclear immunostaining but moderate (++) cytoplasmic staining. **G:** Same PTC (magnification, ×40). Note the completely absent (−) staining in the nucleus compared to the cytoplasm. **H:** UTC (magnification, ×10) with heterogeneous PTEN immunostaining pattern. Islands of immunopositivity interspersed among large areas of immunonegativity. **I:** Same UTC (magnification, ×20). Note that the immunopositive cells are small and round whereas the immunonegative cells are large with large pleomorphic nuclei.

Table 2. Correlation between Intensity of Nuclear PTEN Staining and LOH Analysis

Intensity of PTEN staining	Nucleus		Cytoplasm	
	LOH (%)	No LOH (%)	LOH (%)	No LOH (%)
+++	0 (0)	3 (100)	0 (0)	0 (0)
++	9 (21)	34 (79)	8 (18)	36 (82)
+	12 (32)	26 (68)	15 (36)	27 (64)
−	6 (75)	2 (25)	4 (67)	2 (33)

Statistical correlation of PTEN intensity *versus* LOH performed using a Mantel-Haenszel test yielded results of $\chi^2_1 = 8.70$, $P = 0.003$ for nuclear and $\chi^2_1 = 7.11$, $P = 0.008$ for cytoplasmic PTEN staining, indicating a significant increase in LOH at 10q23 with decreasing PTEN stain intensity.

with dichotomous staining intensity and available paired normal and thyroid tissue. One of these four tumors showed LOH whereas the other three tumors did not. Interestingly, in this tumor, LOH was identified in both immunoreactive-positive and -negative regions.

Among the 92 informative tumors assessed for LOH and PTEN immunostaining, there seemed to be an associative trend between decreased or absent staining and 10q23 LOH (Table 2, Figure 3). The proportion of tumors that had LOH steadily increased from tumors with +++ nuclear staining (0% with LOH), ++ staining (21% LOH), + staining (32% LOH) to no (−) staining (75% LOH) ($P = 0.003$). This trend was also mirrored if we considered only cytoplasmic staining and LOH status (++ cytoplasmic staining [18% with LOH], + staining [36% LOH], to no [−] staining [67% LOH]) (Table 2; Figure 3; $P = 0.008$). Two samples showed no PTEN staining without evidence for LOH. One of the samples was heterozygous for IVS8 + 32G/T whereas the other markers were not informative. The other sample showed regions of heterozygosity at D10S579 and was not informative for the other markers.

Discussion

In this study, we have demonstrated that *PTEN* is strongly expressed in normal thyroid epithelium, in particular in the nucleus and to a lesser extent in the cytoplasm. Endothelial cells also show strong PTEN expression with a nuclear predominance. In benign thyroid tumors, the nuclear expression is weaker compared to normal thyroid follicular cells but still remains at relatively high levels whereas the cytoplasmic staining remains unchanged. In contrast, in thyroid carcinomas, nuclear and cytoplasmic *PTEN* expression, as judged by immunohistochemistry, is reduced. The reduced intensity of the nuclear staining often predominated the reduced intensity in the cytoplasm, in particular in FTC and PTC. In some advanced or aggressive malignant tumors, eg, UTCs, no PTEN staining was detectable at all. These data support a role for PTEN in the pathogenesis of nonmedullary thyroid adenomas and carcinomas, although the marked reduction to no PTEN immunostaining as well as the relatively higher percentage of 10q23 deletion in UTCs does argue that PTEN's role in thyroid tumorigenesis may be in tumor progression rather than tumor initiation.

Because PTEN has not been shown to have a nuclear localization signal,[1,2] cytoplasmic expression of PTEN is expected. In normal thyroid cells, as expected, PTEN is strongly expressed. The decrease in PTEN immunoreactivity in the cytoplasm from normal cells to differentiated carcinomas and to UTC supports our hypothesis that PTEN is inactivated and plays a role in thyroid tumorigenesis. Decreasing PTEN expression in this progression from adenoma to UTC would result in poor control of G1 arrest, apoptosis, and/or cell-cell adhesion.[13,15,35,36]

Given that PTEN does not have a nuclear localization signal,[1] our observation of prominent and differential nuclear PTEN staining is puzzling. Our initial postulate that this represented nuclear membrane staining would appear to be more plausible, and such staining could reflect PTEN's role in regard to the cytoskeleton. Other unrelated studies have noted this nuclear staining with different PTEN antibodies, without explanation. A weak or absent intensity of nuclear PTEN staining along with a strong cytoplasmic PTEN staining was also observed in prostate cancer xenografts[37] and fibroblasts.[11] Together with these previous incidental observations, our immunohistochemical evidence of nuclear staining is likely real, given that the decreasing nuclear signal with less-differentiated carcinomas predominates the decreased cytoplasmic staining. The precise mechanism for our observations is yet unknown.

The equally puzzling observation that decreased nuclear staining often precedes the decreased cytoplasmic staining in thyroid cancers is intriguing. Because PTEN *per se* does not have a nuclear localization signal, it is possible that PTEN is shuttled into the nucleus by another molecule. Such a mechanism has been described for the tumor-suppressor TP53 which is shuttled between the nucleus and the cytoplasm via the oncoprotein MDM2.[38] Other examples exist in the literature. Phosphorylated forkhead-related transcription factor is excluded from the nucleus by interaction with 14-3-3.[39] When not bound to 14-3-3, forkhead-related transcription factor enters the nucleus and acts as a transcription factor for various genes, including, presumably, FAS ligand.[40] VHL and β-catenin have also been documented to play different roles depending on their subcellular localization.[41,42] Based on the current state of knowledge, we would speculate that PTEN could be shuttled into the nucleus related to the cell cycle and/or as a response to cell division and cell growth. It is very well known that intracellular substrates can show a distinct distribution within different compartments (eg, cytoplasm, nucleus, microsomes), ie, differential intracellular compartmentalization. Hence, differential compartmentalization of PTEN might play some as yet undefined role in the tumorigenetic process.

As more studies are performed, it is becoming apparent that inactivation of PTEN relies on multiple diverse mechanisms and not merely structural abnormalities such as somatic mutations.[10,43] In the present study, approximately one fourth of FAs and one fourth of the differentiated carcinomas had LOH. Both FAs and FTCs have a similar frequency of LOH as previously noted. The relatively high frequency of LOH in PTCs (21%) and UTCs (59%) is higher than that reported in the literature possibly because we used *PTEN*-specific markers. The markers in other studies were not specifically chosen for 10q23 but rather broadly covered the whole long arm of chromosome 10. Somatic intragenic mutations of *PTEN* are also not likely to play a major role in *PTEN* silencing because they have been rarely found in thyroid carcinomas.[25,27] Another mechanism which has been implicated in prostate cancer and non-Hodgkin's lymphoma is gene silencing by hypermethylation of CpG islands, presumably in the putative promoter,[10,37] although the situation in prostate cancer is still controversial.[33] Finally, at least in hematological malignancies, reduced or absent PTEN protein levels seem to involve either the postranscriptional, translational, or protein degradation pathways.[10] Given our observations, all of these mechanisms likely come into play in the situation of epithelial thyroid tumorigenesis. We also add another possible mechanism: differential compartmentalization of PTEN. These issues need to be addressed and characterized at the functional level in the future.

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